PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference NEB-236-PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US04/39288	International filing date (*day/month/year*) 22 November 2004 (22.11.2004)	(Earliest) Priority Date (*day/month/year*) 21 November 2003 (21.11.2003)
Applicant NEW ENGLAND BIOLABS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 10 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☒ **Certain claims were found unsearchable** (See Box No. II)
3. ☒ **Unity of invention is lacking** (See Box No. III)
4. With regard to the **title**,
   ☐ the text is approved as submitted by the applicant.
   ☒ the text has been established by this Authority to read as follows:

Please See Continuation Sheet

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the drawings to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12, 24
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11,13-16,18-20,22,23,26-28 and 31

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 9/16
US CL : 435/199
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/199, 252.3, 320.1, 7.6; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HADDEN et al. Crystal structure of the Holliday junction resolving enzyme T7 endonuclease I. Nature Structural Biology. January 2001, Volume 8, No. 1, pages 62-67,	1-11, 13-16, 18-20, 22-23, 26-28 and 31
A	ARAVIND et al. Holliday junction resolvases and related nucleases: identification of new families, phyletic districbution and evolutionary trajectories. Nucleic Acids Research. 2000, Volume 28, No. 18, pages 3417-3432.	1-11, 13-16, 18-20, 22-23, 26-28 and 31
X,P --- Y,P	GUAN et al. Changing the Enzymatic Activity of T7 Endonuclease by Mutations at the beta-Bridge Site: Alteration of Substrate Specificity Profile and Metal Ion Requirements by Mutation Distant from the Catalytic Domain. Biochemistry. April 2004, Volume 43, pp. 4313-4322, entire document.	1-11, 13-16, 18-20, 22-23, 31 --- 26-28
Y	PARKINSON et al. The Junction-resolving Enzyme T7 Endonuclease I: Quaternary STructure and Interaction with DNA. Journal of Molecular Biology. 1997, Volume 270, pages 169-178, entire document.	1-11, 13-16, 18-20, 22-23, 26-28 and 31
Y	DUCKETT et al. Binding of the junction-resolving enyzme bacteriophage T7 endonuclease I to DNA: separation of binidng and catalysis by mutation. journal of Molecular Biology. 1995, Volume 246, pages 95-107, entire document.	1-11, 13-16, 18-20, 22-23, 26-28 and 31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 July 2005 (25.07.2005)

Date of mailing of the international search report
11 AUG 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Kathleen Kerr

Telephone No. 571-272-0700

Form PCT/ISA/210 (second sheet) (January 2004)

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Continuation of Item 4 of the first sheet:
The Title is not short and precise. The Examiner suggests the following title: Modified DNA Cleaving Enzymes and Methods of Use

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-11, 13-16, 18-20, and 22-23, 26-28 and 31, drawn to a modified DNA cleaving enzyme comprising at least the protein encoded by gene 3 (enterobacteria phage T7/T7 endodeoxyribonuclease I, SEQ ID NO: 1); related nucleic acids, vectors and host cells; methods of expressing said nucleic acids; and a first method of use involving determining whether a DNA substrate has a single nucleotide polymorphism (SNP) by contacting the substrate with at least the enzyme encoded by gene 3.

Group II, claim(s) 1-11, 13-16, 17, 21-23, 31 drawn to a modified DNA cleaving enzyme comprising at least Yersenia pestis phage phiA1122 (SEQ ID NO: 13), related nucleic acids and host cells and methods of expressing said nucleic acids.

Group III, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Phage Phi Ye03-12 endonuclease.

Group IV, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Phage T3 endonuclease (phage T3 endodeoxyribonuclease).

Group V, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Pseudomoas phage gh-1 endonuclease.

Group VI, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Pseudomoas putida KT2440 endodeoxyribonuclease I.

Group VII, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Roseophage S101 RP endonuclease I.

Group VIII, claim(s) 25, drawn to a method for modifying enzyme catalytic activity involving selecting an enzyme having two catalytic centers connected by a β-bridge.

Group IX, claim(s) 29, drawn to a method of forming a shotgun cloning library involving incubating a modified DNA cleaving enzyme with a DNA to form non-sequence specific cleavage gragments of the DNA that are ligatable.

Group X, claim(s) 30, drawn to a method for mapping nicks in a duplex DNA involving incubating a modified DNA cleaving enzymes with the duplex DNA in a manganse buffer and permitting nicking to occur.

The inventions listed as Groups I-X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The special technical feature of Group I is a DNA cleaving enzyme with at least 35% identity to T7 Endo I (gene 3, enterobacteria phageT7, SEQ ID NO: 1), two catalytic centers separated by a beta-bridge, and at least one mutation in the beta-bridge that effects enzyme cleavage activity. Nucleic acid molecules encoding said enzymes and host cells containing said nucleic acid molecules can also

be characterized by this special technical feature. Methods of expressing said nucleic acid molecules are characterized as the first method of making the product of the special technical feature. Methods of determining whether a DNA substrate has a single nucleotide polymorphism involving contacting the substrate with at least gene 3 are characterized as the first methods of using the product of the special technical feature.

The products of Groups II-VII, drawn to modified DNA cleaving enzymes comprising at least particular polypeptides (i.e. Yersenia pestis phage phiA1122 (SEQ ID NO: 13), Phage PhiYe03-12 endonuclease, etc.) do not share a special technical feature with Group I because they are drawn to enzymes from different organisms/viruses with different structural features (i.e. different amino acid sequences).

Groups VIII-X are drawn to additional methods of making and using modified DNA cleaving enzymes that do not specifically use the special technical feature of Group I (modified T7 Endo I).

Continuation of B. FIELDS SEARCHED Item 3:
STN, WEST: (ENDODEOXYRIBONUCLEASE OR ENDONUCLEASE OR ENDORIBONUCLEASE or RESOLVASE OR (RESOLVING ENZYME)) and (MUTA? or (MUTA? AND BRIDGE) or (structure and muta? and activity)); SEQ ID NO: 1 in DNA Databases (Patent and Non-Patent)